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Periostin expression upregulated in leiomyosarcoma

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Abstract
Aim: Uterine leiomyosarcomas (LMSs) are rare tumors of the uterus and constitute 1-2% of uterine malignancies. Due to metastases, high recurrence rate and poor prognosis of these tumors, the search for new biomarkers for early diagnosis has increased. In our study, we aimed to show the gene expression and protein level of periostin in leiomyosarcoma as a new biomarker in order to predict prognosis and treatment, together with early diagnosis in leiomyosarcoma.
Material and Methods: Between 2010 and 2020, 12 patients diagnosed with "leiomyosarcoma" and 13 patients diagnosed with "myoma" in our tertiary care hospital, after histopathological examination of clinical samples, were included in the study. Tumor cell necrosis, cellularity, atypia and mitotic index findings of each patient diagnosed with LMS were examined, and staging was performed using the 2009 International Federation of Gynecology and Obstetrics (FIGO) classification system. The tumor preparations of the cases were taken from the pathology archive and re-evaluated, and the gene expression and protein level of periostin were studied in appropriate blocks using ELISA and PCR methods.
Results: According to the FIGO classification, four patients were Stage 1a, six patients were Stage 1b, one patient was Stage 2a, and one patient was Stage 2b. It was determined that the periostin protein was mainly localized in the cytoplasm of leiomyosarcoma cells, and the expression of the periostin gene was increased in cancer tissues compared to myoma tissues, but the difference was not significant (2.16±1.79 vs. 4.01±2.84; p=0.062). Although the mean protein level in periostin tissues was higher in leiomyosarcoma cases, the difference was not significant (LMS: 1.69±1.52; Myoma: 0.88±0.93; p=0.115).
Discussion: Our study is the first to examine the relationship between periostin and leiomyosarcoma. The findings of our study show that periostin can be used as a biomarker for leiomyosarcoma.

Keywords
Leiomyosarcoma, Periostin, Myoma.

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Introduction

Uterine leiomyosarcomas (uLMSs) are rare tumors of the uterus and constitute 1-2% of uterine malignancies [1]. Since these tumors are associated with metastases, high recurrence rate, and poor prognosis, mitotic index, patient age, lymphovascular space invasion (LVSI), and tumor size are evaluated as prognostic biomarkers in high-grade uLMS [2]. Although uLMS is the most common uterine sarcoma, it is difficult to distinguish these tumors from benign leiomyomatous diseases with myometrial localization and preoperative imaging. It is thought that MR and newly developed tissue analysis methods will be more important in the diagnosis with preoperative imaging and in distinguishing the benign or malignant degeneration border of putative fibroids [3]. The gold standard treatment for uLMS is surgical excision with negative margins. If the specimen sent during operation is known to be malignant as a result of the frozen examination, total hysterectomy is preferred. However, mostly, uLMS is diagnosed post-operatively after surgical excision, assuming benign leiomyomatous disease [4]. In studies and follow-ups, adjuvant treatments have not been shown to be beneficial in survival in uLMS, and biomarkers of early diagnosis and response to treatment have not yet been determined [5].

Periostin (POSTN), also known as osteoblast-specific factor 2, first identified in murine osteoblast-like cells in 1993, is a member of the fasciclin protein family [6]. Periostin has been found to be expressed at different levels in multiple solid tumor-associated malignancies. Studies show that periostin can induce tumor angiogenesis, invasion, and metastasis, thus leading to the emergence and progression of malignancies [7]. Yang et al. showed in their meta-analysis that patients with high levels of periostin expression had a worse prognosis than those with no or low levels of expression [8]. Particularly, it was observed that the expression of periostin in tumor tissues was significantly overexpressed compared to precancerous or normal tissues, and a correlation was observed between high periostin levels and undifferentiated tumor differentiation, microvascular invasion, and lymph node metastases [8].

In our study, we aimed to show the gene level and protein expression of periostin in uLMS as a new biomarker to predict prognosis and treatment, together with early diagnosis.

Material and Methods

Patients

The study included 12 patients whose clinical samples were diagnosed with "leiomyosarcoma" after histopathological examination and 13 patients diagnosed with "myoma" in our tertiary care hospital between 2010 and 2020. Detailed pathology reports and clinical information of the cases were obtained by scanning the archive. As a result of the information obtained, it was seen that the patients underwent either myomectomy or hysterectomy and bilateral salpingo-oophorectomy, as well as pelvic and/or paraaortic lymphadenectomy operations, for example, according to the results of the frozen examination. Tumor cell necrosis, cellularity, atypia and mitotic index findings of each patient diagnosed with LMS were examined and staging was performed using the 2009 International Federation of Gynecology and Obstetrics (FIGO) classification system [9]. The tumor preparations of the cases were taken from the pathology archive and re-evaluated, and the gene expression and protein level of periostin were studied in appropriate blocks using ELISA and PCR methods.

Ethics

The study was approved by the Ethics Committee of Zeynep Kamil Women's and Children's Diseases Training and Research Hospital. All patients consented to treatment according to institutional guidelines, with informed consent. All patients consented to anonymous evaluation and analysis of data and treatment outcomes. This study was carried out in accordance with the Declaration of Helsinki and the guidelines of Zeynep Kamil Women's and Children's Diseases Training and Research Hospital Ethics Committee.

Gene expression of Periostin by qRT-PCR

From each paraffin block, 5 tissue sections (each 10-µm thick) were collected into 1.5-ml microfuge tubes. Extraction of total RNA from paraffin-embedded tissues was determined in duplicate using FFPE RNA isolation kit (Invitrogen; Catalogue number K156002) according to the manufacturer's recommendation. One µg RNA was reverse transcribed to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystem) according to the manufacturer's instructions. Amplification was performed with an ABI StepOne Plus detection system using a SYBRGreen PCR Master Mix (Applied Biosystem). The reaction conditions were as follows: 95°C for 10 min, then 40 cycles of 95 °C for 15 sec, 60°C for 1 min. The results were analyzed using StepOne Software v2.3 (Applied Biosystems, Foster City, CA), and normalized by GAPDH as an internal control. Data were expressed as fold induction relative to the control.

Protein Levels of Periostin by ELISA

Formalin-fixed paraffin-embedded 4 tissue sections (each 10–15-µm thick) were collected into a 1.5 ml centrifuge tube. Samples were incubated in 250 µl buffer (pH 7.5, 0.05 M Tris, 1 mM EDTA, and 0.5% Tween 20). Protein extraction of all samples was performed as described previously [10]. Protein concentrations were measured with the Bradford method [11]. Periostin levels were measured with the sandwich enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's protocols (Fine Test; Catalogue number EH0255) with inter-assay cv: <12% and intra-assay cv: <10%, respectively. The mean minimum detectable quantity of human Periostin was 0.094ng/ml. Periostin values were presented as gn/µg protein. All ELISA measurement was performed using a microplate reader was used (BioTek Epoch, Winooski, VT, USA). Results were given as milliliter per milligram of protein.

Histopathological evaluation

After fixation, samples were embedded in paraffin blocks and cut into 5 µm thick sections using a Leica RM2125RTS microtome device (Leica Biosystems, Nussloch, Germany). Selected paraffin sections were stained with hematoxylin-eosin (H&E) staining for morphological evaluation, and the remaining sections were used for immunohistochemistry. All slides were examined under a light microscope (Olympus BX-51, Olympus, Tokyo, Japan).

Statistical analysis

Data are shown as the mean±standard error from at least three independent experiments. One-way ANOVA was applied

to evaluate differences among the multiple groups. Student's t-test was used to perform the statistical comparisons between the two groups. If the variances were homogeneous, the two groups were compared using the least significance difference (LSD) method. Otherwise, Dunnett's T3 method was included to analyze nonhomogeneous variances between the two groups. All statistical tests were two-sided, and P<0.05 (*) and P<0.01 (**) were considered statistically significant. All analyses were performed using SPSS version 18.0 (IBM, Chicago, IL).

Results

According to the FIGO classification, four patients diagnosed with LMS were Stage 1a, six patients were Stage 1b, one patient was Stage 2a, and one patient was Stage 2b.

Although the mean POSTN gene expression level was higher in the patient group, the difference was not significant (2.16±1.79 vs. 4.01±2.84; p=0.062). Although the mean POSTN protein expression levels were higher in the patient group, the difference was also not significant (1.69±1.52 vs. 0.88±0.93 ng/µg protein; p=0.115) (Table 1) (Figure 1).

In the correlation analysis, it was determined that there was a positive correlation between POSTN protein expression and POSTN gene expression (r=0.879; p<0.001).

In the correlation analyzes performed, no significant correlation was found between POSTN protein expression and hematological parameters (p>0.05 for each). However, there was a positive correlation between the POSTN gene expression and red cell distribution width (r=0.414; p=0.020) and the mean platelet volume (r=0.364; p=0.037), and there were no significant correlations between POST gene expression and other hematological parameters (p>0.05 for each) (Table 2).

A positive correlation was found between POSTN protein expression and mitotic index (r=0.338; p=0.049). Likewise, the same positive correlation was found between POSTN gene expression and mitotic index (r=0.478; p=0.008). A positive correlation was found between POSTN gene expression and tumor diameter (r=0.463; p=0.010) and necrosis (r=0.390; p=0.027). There were no significant correlations between POSTN protein and gene expression levels and other parameters (p>0.05 for each) (Table 3).

Table 1. Tissue POSTN gene and protein expression levels in patients.

Biomarker	Group	N	Mean±SE	P value
POSTN/GAPDH	Control	13	2.16±1.79	0.062
	LMS	12	4.01±2.84	
POSTN (ng/µg protein)	Control	13	0.88±0.93	0.115
	LMS	12	1.69±1.52	

SE: Standard error.

Table 2. Correlation between blood parameters and POSTN gene and protein expression in patients.

Variables		POSTN	POSTN/GAPDH
Neutrophil	rho	0.141	0.204
	p	0.251	0.164
Monocyte	rho	0.092	0.081
	p	0.331	0.350
Lymphocyte	rho	0.150	0.242
	p	0.238	0.122
Red blood cell	rho	0.067	-0.098
	p	0.375	0.320
Hemglobin	rho	-0.186	-0.300
	p	0.187	0.072
Hematocrit	rho	0.027	-0.075
	p	0.449	0.361
Mean corpuscular volume	rho	0.320	0.212
	p	0.059	0.154
Mean corpuscular hemoglobin	rho	-0.138	-0.232
	p	0.256	0.132
Mean corpuscular hemoglobin concentration	rho	-0.066	-0.050
	p	0.378	0.407
Red cell distribution width	rho	0.273	.414
	p	0.094	0.020
Platelets	rho	-0.253	-0.181
	p	0.112	0.193
Mean platelet volume	rho	0.282	.364
	p	0.086	0.037

Pearson's correlation coefficient (r), Statistically significant results are in bold type.

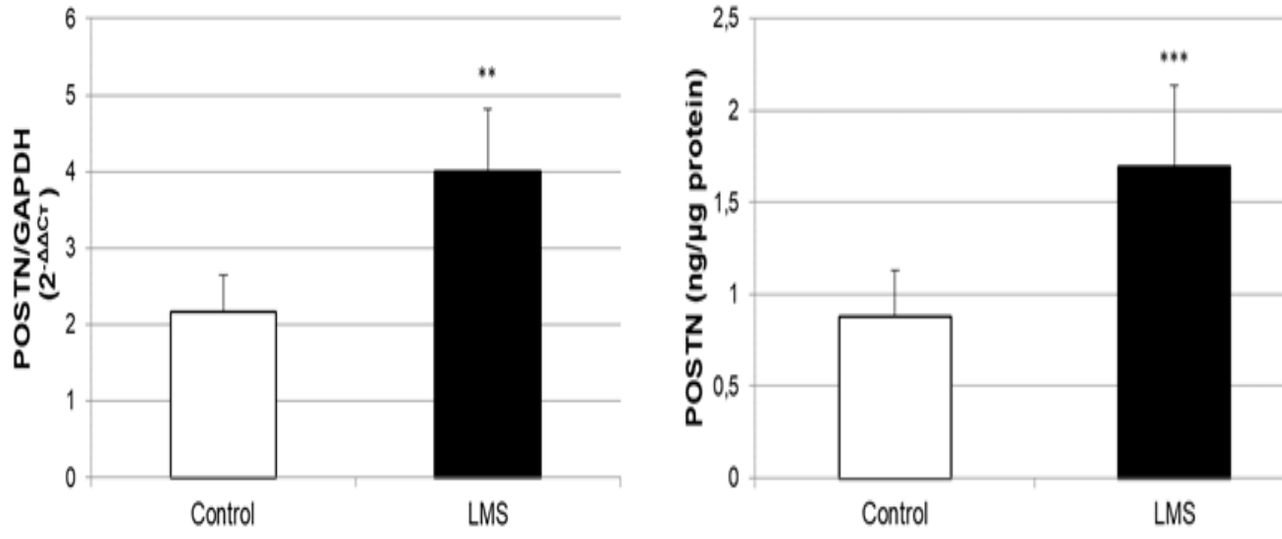

Figure 1. POSTN Gene expression and protein level in leiomyosarcoma and myoma tissues.

Table 3. Correlation between FIGO parameters and POSTN gene and protein expression in patients.

Variables		POSTN	POSTN/ GAPDH
Tumor size	rho	0.295	0.463
	p	0.076	0.010
Grade	rho	-0.015	0.130
	p	0.472	0.268
LVS	rho	-0.166	-0.023
	p	0.213	0.457
Mitose	rho	0.338	0.478
	p	0.049	0.008
A type	rho	0.195	0.273
	p	0.175	0.093
Necrose	rho	0.234	0.390
	p	0.130	0.027

Pearson's correlation coefficient (r), Statistically significant results are in bold type.

Discussion

Uterine leiomyosarcoma, which constitutes approximately 65% of uterine sarcoma cases, is the most common uterine sarcoma [12]. In most uLMS cases, pathological diagnosis based on smooth muscle cell phenotype, cellular atypia, high mitotic index and coagulative necrosis expression is made after tumor resection [13]. uLMS is a malignant tumor with a poor prognosis with a 5-year survival rate of less than 15% in advanced stages, with a recurrence rate of 50-70% after resection [14]. Adjuvant treatments such as chemotherapy, radiotherapy, and hormone receptor blockade are still controversial to reduce relapses and improve survival rates. As a result of our study conducted in uLMS with such a poor prognosis, POSTN protein (2.16±1.79 vs. 4.01±2.84) and gene (1.69±1.52 vs. 0.88±0, 93 ng/µg protein) expression was increased compared to myoma tissue. Our findings show that it can be a marker for early diagnosis of uLMS with rapid progression and metastasis. With this result, our study is the first to examine the relationship between POSTN and uLMS.

It has been found that POSTN is expressed in various normal tissue types such as pancreas, lung, liver, thyroid, stomach, ovary, breast and connective tissue [15]. POSTN, a component of the extracellular matrix (ECM) produced and secreted by fibroblasts, has been shown in studies to interact with various integrin receptors and their signals for differentiation, adhesion and migration regulated by multiple cytokines [16]. Studies have shown that POSTN, whose role is shown in the physiological process, may also be associated with asthma, myocardial damage and cancer, which are pathological processes, with different levels of expression [17]. Tumor invasion and high metastasis capacity are among the most important causes of poor prognosis and decreased survival. Overexpression of POSTN expression has been found to be closely associated with tumor angiogenesis in esophageal squamous cell carcinoma [18]. Siriwardena et al. showed that POSTN protein can increase the formation of tumor local capillaries [19-21]. It is predicted that POSTN interacts with integrin-αvβ3 in malignancy-associated endothelial cells and stimulates the FAK pathway, thereby inducing tumor angiogenesis by regulating VEGR receptor Flk-1/KDR [22]. Some studies have supported the hypothesis that POSTN is involved in lymphatic metastasis and distant metastases of breast cancer [21]. A study on oral squamous cell carcinoma showed that cancer cells expressing high levels of POSTN were more likely to metastasize to the lymph nodes and lungs [22]. In our study, we showed that the gene expression and protein level of POSTN, which are highly expressed in other malignant tumors and are associated with poor prognosis, are increased in malignant tumors with a high local recurrence and metastasis rate such as uLMS [23,24].

Jamaluddin et al. revealed the proteomic profile of uterine fibroids to analyze the ECM protein expression pattern. Using genetic sequencing and isobaric labeled quantitative mass spectrometry (iTRAQ), they analyzed samples in two main groups: MED12 positive and negative mutation (one of the most common anomalies in fibroids). These researchers concluded that POSTN was significantly up-regulated in fibroids regardless of MED12 status [25].

Conclusion

Our study is the first to examine the relationship between POSTN and uLMS. Our results show that POSTN can be used as a biomarker in uLMS. Further and comprehensive studies are needed to fully elucidate the clinical value and prognostic impact of POSTN.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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